The new claims are fully supported by the specification and original claims, and do not introduce new matter. The support for each new claim can be found in the specification, for example, as follows:

new claim number	specification or former claim number
95	figure 12, tester strain
96	74
97	74
98	page 11, lines 16-18
99	page 11, lines 14-16
100	80
101	81
102	page 38, lines 28-31
103	page 69, line 23-page 72, line 22, which describes an inducer (IPTG) that is distinct from the target compound (ascorbic acid)
104	page 38, line 28-page 39, line 4
105	page 12, lines 12-14
106	63, 86, 72
107	74
108	74
109	page 11, lines 16-18
110	page 11, lines 14-16
111	80

112	81
113	page 12, lines 6-9
114	page 38, lines 28-31
115	page 69, line 23-page 72, line 22
116	page 38, line 28-page 39, line 4
117	page 12, lines 12-14
118	86, 88, 89, 90, 91
119	87
120	72, 73, 75, 76, 77
121	74
122	80
123	page 30, lines 30-33
124	page 16, lines 4-7
125	82, 83

Applicants have set forth above the amendments to the specification and claims in clean form as required under 37 C.F.R. § 1.121 (c)(i).

Rejections under 35 U.S.C. §112, second paragraph

Claims 80 and 93 stand rejected under 35 U.S.C. §112, second paragraph, as allegedly indefinite. The rejection is respectfully traversed for the reasons that follow.

Claims 80 and 93 are alleged to be indefinite due to the recitation of the phrase "said cell is a bacteria." Claims

80 and 93 have been canceled and therefore, the rejection is rendered moot. Although Applicants submit that the term is sufficiently clear and definite in view of the specification, new claims 100, 111, and 122, which contain similar subject matter as canceled claims 80 and 93, read "said cell is a bacterial cell" as suggested by the Examiner. Accordingly, it is respectfully requested that the rejection be removed.

Rejections under 35 U.S.C. §112, first paragraph

Claims 75-79, 82-85, and 88-92 stand rejected under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that Applicants were in possession of the claimed invention at the time the application was filed. The Office Action alleges that the specification discloses only two species of the genus of yiaJ nucleic acids and that the species specifically disclosed are not representative of the genus because the genus is highly variant. In addition, the Office Action alleges that none of the species of the claimed genus in the case of the yiaJ binding promoter is disclosed.

The rejection is respectfully traversed for the reasons that follow. Claims 75-79, 82-85, and 88-92 have been canceled and, therefore, the rejection has been rendered moot. Accordingly, it is respectfully requested that this rejection be removed.

Applicants respectfully submit that the specification provides sufficient description and guidance to convey to one skilled in the art that Applicants were in possession of the claimed invention at the time the invention was filed. It is submitted that the yiaJ genes or YiaJ-responsive promoters recited in new claims 118-125 are sufficiently described in the specification for the reasons that follow.

The Office Action alleges that the specification discloses only two species of the genus of yiaJ nucleic acids and that the species specifically disclosed are not representative of the genus because the genus is highly variant. In *The Regents of the University of California v. Eli Lilly* (43 USPQ2d 1398-1412) the court indicated that Applicants are not required to disclose every species encompassed by a genus; the description of a genus is achieved by the recitation of a representative number of DNA molecules, usually defined by a nucleotide sequence, falling within the scope of the claimed genus. Applicants submit that the specification discloses the sequence of three separate yiaJ genes from three separate species of bacteria. The amino acid sequences of yiaJ from *Klebsiella oxytoca* (SEQ ID NO: 10), *Escherichia coli* (SEQ ID NO: 20), and *Haemophilus influenzae* (SEQ ID NO: 21) are disclosed in the specification and compared in Figure 3. In addition, the nucleotide sequence of yiaJ from *Klebsiella oxytoca* (SEQ ID NO:1) is disclosed and the references for the published nucleotide sequences of *Escherichia coli* (Blattner et al.) and *Haemophilus influenzae* (Fleischmann et al.) are cited in the specification. Furthermore, Figure 3 highlights the extensive degree of homology between these three YiaJ

polypeptides, demonstrating that this genus is not highly variant as stated in the Office Action. Therefore, Applicants submit that the three disclosed yiaJ nucleotide and amino acid sequences provide a representative number of yiaJ sequences for the genus of yiaJ sequences. In addition, given the amino acid sequence of YiaJ proteins from three bacterial species and the high degree of homology between these YiaJ proteins, one skilled in the art would be able to identify YiaJ proteins from other species of bacteria present in databases.

The Office Action further alleges that none of the species of the claimed genus in the case of the YiaJ binding promoter is disclosed. Applicants submit that the specification does disclose the sequence of a YiaJ binding promoter within the 5' region of the yiaK-S operon (SEQ ID NO:19). This promoter is depicted schematically in Figure 13 as P_{yia} in the region before yiaK. In addition, guidance is given in the specification, for example, at page 12, line 24 to page 13, line 9, as to what constitutes a promoter including a description of specific sequences such as the TATA box, capping sequence, CAAT sequence, ribosome binding site, start codon and the like.

Furthermore, in Example 1, on page 65, lines 21 to page 66, line 25, the promoter of the yiaK-S operon, which is YiaJ responsive as depicted in Figure 13, was replaced with a DNA fragment containing the trp-lac hybrid promoter. A 1 kb fragment containing the 5' end of yiaK, including its ribosome binding site, but excluding the promoter sequences of the yiaK-S operon, was used in a ligation reaction (page 66, lines 11-15). Also,

PCR analyses were performed on 12 candidates to verify that the endogenous promoter of the yiaK-S operon (the YiaJ responsive promoter) had been replaced with the inducible $lacI^q$-trc promoter system (page 66, line 22-25). Given the schematic in Figure 13, the sequence of the yiaK-S operon in SEQ ID NO:19 and the guidance in the specification regarding promoter elements, Applicants submit that the specification provides sufficient description of a YiaJ binding promoter to one skilled in the art. Furthermore, the teachings in the specification regarding the location of a YiaJ binding promoter combined with the complete genomic sequences of *E. coli* and *H. influenzae* which were incorporated by reference, would allow one skilled in the art to identify a YiaJ binding promoter in *E. coli* and *H. influenzae*.

Applicants respectfully submit that the specification provides sufficient description and guidance to convey to one skilled in the art that Applicants were in possession of the claimed invention at the time the application was filed. Therefore, Applicants respectfully request that these grounds of rejection be withdrawn.

Claims 75-79, 82-85, and 88-92 also stand rejected under 35 U.S.C. § 112, first paragraph, as allegedly lacking enablement. The Office Action alleges that the function of the nucleic acids in the claimed methods is not known and that determining the binding of YiaJ to a promoter and the transcriptional activation of a reporter gene would require undue trial and error experimentation.

The rejection is respectfully traversed for the reasons that follow. Claims 75-79, 82-85, and 88-92 have been canceled and, therefore, the rejection has been rendered moot. Accordingly, it is respectfully requested that this rejection be removed.

Applicants respectfully submit that the specification provides sufficient description and guidance to enable the claimed methods. It is submitted that the yiaJ genes or YiaJ-responsive promoters recited in new claims 118-125 are sufficiently enabled by the specification for the reasons that follow.

The Office Action alleges that the function of the nucleic acids in the claimed methods is not known. The Examiner does acknowledge that the claimed methods are enabled when the one or more genes encode the full yia operon SEQ ID NO:19 and the *E.coli* full yiaK-S operon, which are involved in the metabolism of ascorbate. The function of the yiaJ nucleic acids are also taught in the specification. The function of the yiaJ nucleic acids, as stated in the specification, is to produce a protein, YiaJ, that is transcriptionally activated by the presence of ascorbate, either directly or indirectly. At the priority date of the subject application, it was known that YiaJ was a transcriptional regulatory protein. Using insertional mutagenesis, Badia et al. demonstrated that *E. coli* YiaJ was a transcriptional regulatory protein (Badia et al. J. Biol. Chem. 273:8376-8381 (1998)). As noted in Badia et al., YiaJ was known to contain a helix-loop-helix region which is a protein motif

common to several DNA binding transcriptional regulatory proteins. All three YiaJ proteins disclosed in the specification also contain this DNA binding motif (see Badia et al. *supra* and Figure 3). Furthermore, in a recent paper, gel mobility shift assays were used to show the specific region of the promoter that was bound by *E. Coli* YiaJ (Ibanez et al., J. Bacteriol. 182:4617-4624 (2000) provided as Exhibit A).

The specification teaches that YiaJ is a transcriptional regulatory protein that can respond to ascorbate. This response can be direct, through binding ascorbate, or indirect (see page 74, lines 5-10). For example, as stated in the specification on page 37, lines 20-23, based on transcriptional analyses, the YiaJ regulatory protein is thought to activate transcription of the yiaK-S operon in response to ascorbic acid in the medium. Furthermore, as stated in the specification on page 37, line 31 to page 38, line 3, in the presence of ascorbic acid, YiaJ is stimulated and activates transcription of the yia-GFP fusion, thereby conferring an easily detectable GFP-positive or fluorescent phenotype. Therefore, the function of YiaJ, as stated in the specification, is as a transcriptional regulatory protein.

The Office Action further alleges that undue experimentation would be required to determine the function of a yiaJ nucleic acid and whether or not yiaJ binds to a promoter in a manner that regulates the expression of a reporter gene. The Examiner states that the function of a protein can not be inferred by homology unless the homology is 100%. However, one

of skill in the art can use routine methods well known in the art to determine the transcriptional regulatory function of a YiaJ protein as stated in the specification. For example, one skilled in the art could generate the construct shown in Figure 13B with the P_{yia} linked to a reporter gene using standard recombinant DNA methods. This construct can be transformed or transfected into cells containing YiaJ using routine techniques well known in the art. The expression of the reporter gene in the presence or absence of ascorbate is then determined again using protocols well known to one skilled in the art.

Claims 72-74 also stand rejected under 35 U.S.C. § 112, first paragraph, as allegedly lacking enablement. The Office Action alleges that while being enabling for methods of detecting the presence of some compounds, the specification does not provide enablement for methods of detecting the presence, absence and amount of any compound because the genes, promoters and cells would not be known. In addition, the Office Action alleges that undue trial and error experimentation would be required in order to quantitate the amount of a compound.

The rejection is respectfully traversed for the reasons that follow. Claims 72-74 have been canceled and, therefore, the rejection has been rendered moot. Accordingly, it is respectfully requested that this rejection be removed.

Applicants respectfully submit that the specification provides sufficient description and guidance to enable the claimed methods. It is submitted that the new biosensor method

claims 106-117 are sufficiently enabled by the specification for the reasons that follow.

The Office Action alleges that it would require undue experimentation to determine the genes, promoters and cells utilized by claims 72-74. New biosensor method independent claim 106 utilizes genes and cells that are described in the claims as having certain features that are taught in the specification. For example, in new claim 106, the cells contain a pathway from a source compound to a target compound and one or more genes responsible for converting the target compound to a detectable signal. Source and target compounds and genes responsible for generating detectable signals are described throughout the specification. Therefore, the genes and cells utilized in new independent claim 106 and its dependents are described in the specification such that only routine experimentation would be required for one skilled in the art to use the invention as claimed.

The Office Action alleges that undue experimentation would be required in order to quantitate the amount of a compound. In addition, the Office Action questions whether the amount of a particular compound can be based on a particular signal because of sensitivity issues or threshold issues. Applicants submit that routine methods were known in the art for quantitating the amount of a compound and dealing with sensitivity and threshold issues. For example, two of references cited by the Examiner in the Action, Applegate et al. and Selifonova et al., use a reporter gene assay to quantitate the

amount of organic compounds such as benzene and toluene. In these references, growth response curves and standard curves comparing the signal generated to the compound concentration were performed. For example in Applegate et al., the concentration of toluene (mg/L) was compared to specific bioluminescence (namp/OD) to create a dose response curve (see Figure 4). Thus, methods were known in the art for quantitating the concentration of compounds in biosensor methods.

Applicants respectfully submit that the specification provides sufficient description and guidance to enable the claimed methods. Accordingly, Applicants respectfully request that this ground of rejection be withdrawn.

Rejections under 35 U.S.C. §102

Claims 82-84 stand rejected under 35 U.S.C. §102(a) as allegedly anticipated by Badia et al. (reference AK on PTO form 1449, filed 04-25-00). Badia et al. is alleged to describe a nucleic acid encoding an *E. coli* yiaJ and a CAT reporter gene transcriptionally linked to the gene, and cells comprising this nucleic acid. The rejection is respectfully traversed for the reasons that follow. Claims 82-84 have been canceled and, therefore, the rejection has been rendered moot. Accordingly, it is respectfully requested that this rejection be removed.

It is submitted that new claims 118-125 are novel in view of Badia et al. for the reasons that follow. Applicants submit that the Badia et al. reference does not describe a CAT

reporter gene transcriptionally linked to a yiaJ gene, but instead describes inactivation of the yiaJ gene by CAT gene insertion. In this reference CAT was used for insertional mutagenesis of yiaJ instead of as a reporter gene (see Badia et al., page 8379 column 1 paragraph 1). Thus, the reference by Badia et al. does not teach the nucleic acids or cells of the claimed invention and, therefore, it can not anticipate the claimed invention.

Claims 72, 74, 80, 86, 87, and 93 stand rejected under 35 U.S.C. §102(a) as allegedly anticipated by Applegate et al., (reference AJ on PTO form 1449, filed 04-25-00). It is alleged that Applegate et al. describe a method for detecting benzene, toluene, ethylbenzene and xylene using a bacterial strain which comprises genes which produce luminescence in response to the presence of any of these compounds. The rejection is respectfully traversed for the reasons that follow. Claims 72, 74, 80, 86, 87, and 93 have been canceled and, therefore, the rejection has been rendered moot. Accordingly, it is respectfully requested that this rejection be removed.

It is submitted that new claims 95-117 are novel in view of Applegate et al. for the reasons that follow. The Applegate et al. publication does not teach a method for detecting the presence, absence, or amount of a target compound in a cell by providing a recombinant cell where the cell contains an isolated nucleic acid that converts a source compound to a target compound. Furthermore, Applegate et al. do not teach a cell that contains an isolated nucleic acid molecule responsible

for converting a source compound to a target compound and one or more isolated genes responsible for converting the target compound to provide a detectable signal. Thus, the publication by Applegate et al. does not teach the methods or cells of the claimed invention and, therefore, it can not anticipate the claimed invention.

Claims 72, 74, 80, 86, 87, and 93 stand rejected under 35 U.S.C. §102(b) as allegedly anticipated by Selifonova et al. (reference BV on PTO form 1449, filed 04-25-00). It is alleged that this publication describes a method of detecting certain hydrophobic pollutants using a bacterial strain which comprises genes which produce a luminescent signal in response to the presence of these compounds. The rejection is respectfully traversed for the reasons that follow. Claims 72, 74, 80, 86, 87, and 93 have been canceled and, therefore, the rejection has been rendered moot. Accordingly, it is respectfully requested that this rejection be removed.

It is submitted that new claims 95-117 are novel in view of Selfonova et al. for the reasons that follow. The Selifonova et al. publication does not teach a method for detecting the presence, absence, or amount of a target compound in a cell by providing a recombinant cell where the cell contains an isolated nucleic acid that converts a source compound to a target compound. Furthermore, Selfonova et al. do not teach a cell that contains an isolated nucleic acid molecule responsible for converting a source compound to a target compound and one or more isolated genes responsible for converting the target

compound to provide a detectable signal. Thus, the publication by Selifonova et al. does not teach the methods or cells of the claimed invention and, therefore, it can not anticipate the claimed invention.

Claims 72, 74, 80, 81, 86, 87, 93, and 94 stand rejected under 35 U.S.C. §102(e) as allegedly anticipated by U.S. Patent No. 5,989,832 by Trias et al. It is alleged that Trias et al. describe methods for the detection of the presence of a non-tetracycline-specific efflux pump inhibitor using bacterial cells which produce the signal of inhibited growth in the presence of the inhibitor. The rejection is respectfully traversed for the reasons that follow. Claims 72, 74, 81, 80, 86, 87, 93 and 94 have been canceled and, therefore, the rejection has been rendered moot. Accordingly, it is respectfully requested that this rejection be removed.

It is submitted that new claims 95-117 are novel in view of Trias et al. for the reasons that follow. The Trias et al. patent does not teach a method for detecting the presence, absence, or amount of a target compound in a cell by providing a recombinant cell where the cell contains an isolated nucleic acid that converts a source compound to a target compound. Furthermore, Trias et al. do not teach a cell that contains an isolated nucleic acid molecule responsible for converting a source compound to a target compound and one or more isolated genes responsible for converting the target compound to provide a detectable signal. Thus, the patent by Trias et al. does not

teach the methods and cells of the claimed invention and, therefore, it can not anticipate the claimed invention.

CONCLUSION

In light of the Amendments and Remarks herein, Applicants submit that the claims are now in condition for allowance and respectfully request a notice to this effect. Should the Examiner have any questions, she is invited to call Cathryn Campbell or the undersigned agent.

Respectfully submitted,

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Date

Melanie K. Webster
Registration No. 45,201
Telephone: (858) 535-9001
Facsimile: (858) 535-8949

CAMPBELL & FLORES LLP
4370 La Jolla Village Drive
7th Floor
San Diego, California 92122
USPTO CUSTOMER NO. 23601